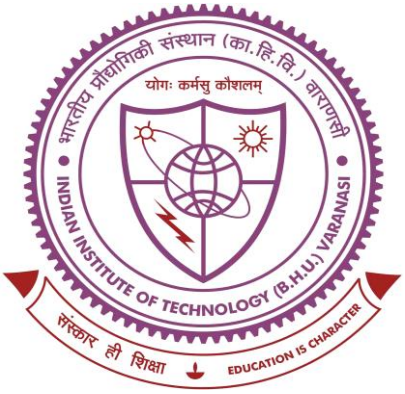
Optimization and Characterization Of Biosurfactant Produced from an Acclimatized Strain *C. tropicalis* MTCC230, Its Application and *Insilico* Studies



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CHAPTER 7

Summary of work

7.1 Summary of the Work

Biosurfactants are surface-active amphiphilic metabolites compounds produced by living surfaces, mostly on microbial cell surfaces excreted extracellularly. They interact with the phase boundary between two phases in a heterogeneous system and enhance the emulsification of hydrocarbons to solubilize hydrocarbon contaminants and increase their availability for microbes. They have wide application in chemical, food, medical, cosmetic, agriculture and pharmaceutical industries,

In number of studies it was found that the survival chances could become lesser of genetically modified microorganisms when they were introduce under stress environmental condition such as crude oil contaminated soil. To overcome this problem one of the best approach is to adapt or acclimatize the microbial strain under stress environmental (hydrocarbon) condition for the production of biosurfactant. It has been reported that yeast shows higher production of biosurfactant than bacteria this is due to presence of a rigid cell wall. In prokaryotic cells, the membrane may be damaged at high concentrations of biosurfactant. Among yeasts, *Candida sp.* has been widely used for Biosurfactant production when grown on water-immiscible substrates

In this work *Candida tropicalis* MTCC 230 get acclimatized under high concentration of hydrocarbons, for petrol (0.5%glucose+1.5%petrol) for kerosene oil (0.5%glucose+1.5% kerosene) and for mustard oil (0.75% glucose+0.75% mustard oil). Best microbial growth profile was obtained when 0.5%glucose+1.5%petrol oil was used as carbon for *Candida tropicalis* MTCC230 and also showing highest emulsification index. Estimation of emulsification index is the direct method to determine the biosurfactant

production when immiscible substrate was used as a carbon source. All the hydrocarbons tested served as substrates for emulsification by the biosurfactant but petrol oil was the best substrate while waste kitchen oil (mustered oil) was the poorest. The optimum pH and temperature was 6.8 and 34^0C respectively, for highest cell growth under acclimatized hydrocarbon condition.

Ammonium chloride, ammonium nitrate and sodium nitrate were used as a nitrogen source for biosurfactant production from an acclimatized *Candida tropicalis MTCC230*. , among these Ammonium chloride shows the highest Emulsification index (% E_{24}) this shows that ammonium chloride served as a best nitrogen source for an acclimatized *Candida Tropicalis MTCC230* to produce emulsifier for solubilization of immiscible carbon source. Micro and trace elements play a major role for biosurfactant production from an acclimatized *C.tropicalis* MTCC230. When they were used all together in the production media the highest microbial growth and emulsification index (E_{24}%) was obtained.

Candida tropicalis MTCC 230 used to acclimatize in hydrocarbon along with glucose for biosurfactant production, showing diauxic growth during the production. Process parameters were optimized one factor at a time, showing highest emulsification index (%E_{24}) of 54%. The production of biosurfactant was enhanced by using biostatical based experimental design with the interactive effect of different parameters. On the basis of Placket-Burman design four factors, hydrocarbon, ammonium chloride, microelements and temperature are found significant ($P<0.05$) for the production of biosurfactant. A second order polynomial regression model in central composite design estimated the maximum biosurfactant production in terms of emulsification index (%E_{24}). The optimum

combination of different parameters for the biosurfactant production, obtained for hydrocarbon, ammonium chloride, microelements and temperature are 81.41%, 1.63(g/l), 1.69(g/l) and 35.25ºC, respectively. The biosurfactant production was increased by two fold after optimization and selection of interactive parameters by response surface methodology.

Biosurfactant produced from an acclimatized strain *C. tropicalis* MTCC230 was characterized as surfactin, showed similar functional groups and wave number position in FTIR and Near-IR spectrum as shown by surfactin. HPLC chromatograms of standard surfactin (Sigma-Aldrich) also share the similar chromatogram peak with similar retention time at approx. 4.3 to 6.8 minute as shown by surfactin (Sigma-Aldrich). Result of Mass spectrometry (MS) analysis revel that The intact mass of singly charged and doubly charged was 1036 dalton and 518 dalton respectively of biosurfactant produced from an adaptive strain *C. tropicalis* MTCC230 that was exactly the same mass of surfactin standard surfactin (Sigma-Aldrich). These results confirmed that biosurfactant produced from an acclimatized strain *Candida tropicalis* MTCC 230 was considered as surfactin class of lipopeptide biosurfactant.

Oil spreading method and soil washing analysis confirmed that the biosurfactant produced from a *C. tropicalis* MTCC230 showing the promising results in the field of bioremediation to remove the hydrocarbon contaminants from water and soil. It can significantly reduce the surface tension 72 mN/m to 32mN/m, with the critical micelle concentration (CMC) of 32.5mg/l. It shows stable Physiochemical properties under the extreme pH (2-12), temperature (30°C-90°C) and salinity (2%- 10%) that promote its potential use in enhanced oil recovery. Application of biosurfactant produced from *C.*

tropicalis MTCC230 in enhanced Oil recovery (EOR) was done by laboratory scale sand pack column saturated with four stroke engine oil resulted 39.80% additional oil recovery (AOR), suggesting its suitability for microbial enhanced oil recovery (MEOR) in oil reservoirs.

Insilico study was done where surfactin used as a ligand (drug) and amyloid β - peptide (Aβ42) was target protein. Aggregation of amyloid b-peptide (Aβ42) into fibrils is a key pathological process associated with Alzheimer's disease. In this study, effect of surfactin against amyloid β peptide was studied by using computational approaches. In the molecular docking, surfactin interacts with A chain of amyloid fibril and forms the hydrogen bonds with Ala 21 and Asp 23 with total energy of -3.28 kcal/mol. Surfactin interacts with an amphiphilic pore amyloid b-peptide (Aβ42); binding of surfactin to amyloid fiber shows the decrease in salt bridge length (between Asp 23 and Lys 28) from 11.5 to 9.0 A°; and this may lead to displace the water molecules and so destabilize the amyloid b-peptide (Aβ42). 10-ns molecular dynamics simulation was performed for amyloid fibril and with surfactin amyloid fibril complex. RMSD, RMSF, Rg trajectories, and SASA plot further used to study the stability of complex and effect of surfactin.

This study has shown that how single molecule of Surfactin (lipopeptide) interact with Aβ fibril and destabilize it. This work has explored the effects of deposition of surfactin on the surface of the protofibril, and its potential to penetrate into the hydrophobic core and amphiphilic pore to displace the water molecule which results destabilization of protofibril, and the ability of surfactin to prevent the deposition of an incoming Aβ peptide to the preformed protofibril. This in silico study of surfactin against

the Aβ amyloid fibril responsible for Alzheimer provides information for furthering drug design for the treatment of Alzheimer's disease in the future.

Respiratory distress syndrome (RDS) is caused by a deficiency of Lung surfactant and they are more prominent in premature infant. Biosurfactant can be considered for RDS treatment as it clear the primarily study of radiological property of drug by comparable study with FDA approved drug survanta, showing the same IR pre-clinical spectrometric results. Animal derived surfactants and chemically synthesized surfactants used for RDS treatment having some limitations like limited scale of production when animally derived and hazardous when chemically synthesized, over these limitations microbial synthesized surfactants having some advantages, it can be produced in large scale and found to be non-hazardous for human body. The purpose of this work is to show preclinical radiological study of microbial derived surfactants for respiratory distress syndrome (RDS) treatment. Two spectral diagnostic windows for deep tissue imaging in Near IR spectrum (300-1100nm) and for Far IR spectrum (1200nm-2200nm) were used for comparable study of FDA approved drug survanta with microbial produced Surfactin and Lipopeptide (Biosurfactant) from *B. subtilis* MTCC2423 and *C.tropicalis* MTCC230 respectively. Absorbance peak for surfactin and lipopeptide (Biosurfactant) was fall in same wavelength as survanta shows *i.e*, 396nm and 967nm. Far IR spectrum (1200nm-2200nm) shows that the least absorbance frequency was obtained at 1249nm for all the three surfactants. Absorbance unit in highest dilution for surfactin, lipopeptide (Biosurfactant) supernatant and survanta were 0.17, 0.24 and 0.42AU respectively. Surfactin and Lipopeptide (Biosurfactant) produced from *B. subtilis* MTCC2423 and *C. tropicalis* MTCC230

respectively, can be considered for RDS treatment as it clear the primarily study of radiological property of drug. This work explore the potential use of microbial synthesized lipopeptide for the treatment of RDS as it passes the pre-clinical trial of radiological study and so this information and data open the path for clinical and *invivo* study of microbial derived surfactant for RDS treatment in neonates.